and a carrier.

REMARKS

Applicants have canceled claims 43 and 49 without prejudice and have added new claims 50 and 51, corresponding thereto, respectively, to introduce certain format changes. Applicants maintain that the addition of new claims 50 and 51 does not raise an issue of new matter. Applicants have also introduced certain format changes to the specification. A marked-up version of the amended paragraphs is annexed hereto as Exhibit H. Accordingly, new claims 50 and 51 are pending in the subject application.

In view of the arguments set forth below, applicants maintain that the Examiner's objections and rejections made in the November 23, 2001 Office Action have been overcome, and respectfully request that the Examiner reconsider and withdraw same.

The Claimed Invention

This invention provides an isolated peptide encoded by a nucleic acid which is at least 30 nucleotides in length and which has a nucleotide sequence that uniquely defines a herpesvirus associated with Kaposi's sarcoma, which herpesvirus is present in and recoverable from the HBL-6 cell line. This invention also provides a composition which comprises the instant isolated peptide and a carrier

Formalities

Claim Objection

Examiner objected to claim 43 because of the following informality: line 4 recites the term "Kaposis'". The Examiner states that the correct term is "Kaposi's".

Applicants have hereinabove canceled claim 43 and replaced it with new claim 50, reciting the correct term "Kaposi's". Accordingly, applicants respectfully request that the Examiner withdraw the objection.

Information Disclosure Statement

The Examiner stated that a copy of U.S. Patent No. 5,306,709 was submitted with applicants' October 9, 2001 IDS and was listed on the transmittal letter. However, the Examiner stated that this patent was not listed on the PTO-1449 form, and requested that applicants clarify that this patent is to be included with this IDS.

In response, applicants attach hereto as Exhibit A a PTO-1449 form listing U.S. Patent No. 5,306,709.

The Examiner also stated that the document of Saiag, et al. (Ann. Der. Ven. 122:551-557 1995) was not considered because it is in the French language and no English language abstract or explanation of relevance was submitted with the document.

In response, applicants attach hereto as Exhibit B an English translation of Saiag, et al. Applicants respectfully request

that both U.S. Patent No. 5,306,709 and Saiag, et al. be made of record.

Sequence Listing

The Examiner stated that this application fails to comply with the requirements of 37 C.F.R. §1.821-§1.825 because the sequences in Figure 6 and the sequences on page 148 are not identified with SEQ ID NOs. The Examiner further stated that if these sequences are not included in the originally filed Sequence Listing they must now be included in a substitute Sequence Listing (both in computer-readable and paper forms). In addition, Examiner stated that the originally filed paper copy of the Sequence Listing is missing pages 214, 216, 219 and 224.

In response, applicants attach hereto as Exhibit C a paper copy of the substitute Sequence Listing. Applicants also submit a computer diskette containing the substitute Sequence Listing in computer-readable form. A Statement of Compliance stating that the contents of the paper copy and computer-readable form are identical is attached hereto as Exhibit D. Finally, applicants attach hereto as Exhibit E a copy of the Notice to Comply. Applicants verify that these submissions do not involve any issue of new matter. Thus, the subject application is in compliance with 37 C.F.R. §1.821-§1.825.

Drawings

In the Notice of Draftperson's Patent Drawing Review issued concurrently with the November 23, 2001 Office Action, the Draftperson made certain objections to the drawings submitted in connection with the subject application.

In response, applicants attach hereto as Exhibit G 48 sheets of new, corrected formal drawings for Figures 1-29F.

Rejections Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 43 and 49 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants understand the Examiner's rejection to apply to new claims 50 and 51 corresponding, respectively, to canceled claims 43 and 49.

In response, applicants respectfully traverse the Examiner's above rejection.

The Examiner asserts that there are no examples shown of any specific 30-nucleotide sequence in the entire genome of a herpesvirus associated with Kaposi's sarcoma. The Examiner also asserts that the disclosure of many sequences of a herpesvirus associated with Kaposi's sarcoma is not descriptive of the complete structure of a representative number of species encompassed by the claims, as one of skill in the art cannot envision the complete structure of any 30-nucleotide sequence based on the disclosed sequences.

Applicants maintain that the Examiner's position is flawed. The claimed invention is drawn to an isolated peptide encoded by a nucleic acid which is *at least* 30 nucleotides in length. Indeed, as the Examiner concedes, there are "many", i.e., no less than 15, amino acid sequences set forth, translated from complete

ORFs, that are specific KSHV polypeptides (see, e.g., Figure 12, ORFs 21-28, 29A, 29B and 30-34). Each of these peptides is encoded by a nucleic acid of at least 30 nucleotides in length.

It is stressed that *no* peptide encoded by a 30-nucleotide-long nucleic acid need be separately listed in order to satisfy the written description requirement. Rather, there already exists a description of a vast array of species of the claimed peptide in Figure 3A (i.e., SEQ ID NO:1). Based on the requisite skill in the art, the separating out and listing of *each* peptide encoded by the nucleotide sequence of Figure 3A which is at least 30 nucleotides in length and uniquely defines a herpesvirus associated with Kaposi's sarcoma would demand a degree of description which is above and beyond that which is minimally required.

The Examiner also states that, additionally, there is no description of a representative number of species by partial structure and a function which correlates with structure.

In response, applicants point out that under M.P.E.P. §2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by disclosure of *relevant, identifying characteristics*. Partial structure and a function which correlates with structure are merely examples of such relevant, identifying characteristics. M.P.E.P. §2163 also states that, in the case of biomolecules, relevant, identifying characteristics include *sequences*. Further, satisfactory disclosure of a "representative number" depends on whether one of ordinary skill in the art would recognize that the applicant was in possession of the necessary common attributes or features

of the elements possessed by the members of the genus in view of the species disclosed. Clearly, one of ordinary skill in the art would recognize that disclosure of a plurality of sequences of at least 30 nucleotides in length in the specification, as applicants have done, constitutes possession of the instant peptide.

The Examiner also rejected claim 43, corresponding to new claim 50, under 35 U.S.C. §112, first paragraph, stating that even though claim 43 and the instant specification indicate that a deposit of the HBL-6 cell line (ATCC Accession No. CRL 11762, page 27) has been made, no statement has been submitted which indicates that all restrictions on availability of the deposited cell line will be removed upon issuance of a patent.

In response, applicants make the following statement:

Cell line HBL-6 (ATCC Accession No. CRL 11762) was received on November 18, 1994, and was accepted, by the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, which is an International Depository Authority recognized under the provisions of the Budapest Treaty. All restrictions upon public access to these deposits will be irrevocably removed upon the grant of a patent on the subject application. The deposit will be replaced if viable samples cannot be dispensed by the ATCC.

In view of the above remarks, applicants maintain that claims 50 and 51 satisfy the requirements of 35 U.S.C. §112, first paragraph.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 43 and 49 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Examiner stated that claim 43 recites the phrase "...has a sequence...", and asserted that it is unclear if the nucleic acid or the peptide defines the herpesvirus.

In response, applicants respectfully traverse the Examiner's rejection, and understand it to apply to new claims 50 and 51. Applicants point out that in new claim 50, the term "sequence" is preceded by "nucleotide", clearly indicating that the term sequence means nucleotide sequence.

In view of the above remarks, applicants maintain that claims 50 and 51 satisfy the requirements of 35 U.S.C. §112, second paragraph.

Summary

In view of the amendments and remarks made herein, applicants maintain that the claims pending in this application are in condition for allowance. Accordingly, allowance is respectfully requested.

If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the $920.00 fee for a three-month extension of time, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231.

5/23/02

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Page 148, lines 1-37:

All samples were tested for amplifiability using primers specific for either the HLA-DQ locus (GH26/GH27) or B-globin [18]. PCR detection fo KSHV DNA was performed as previously described [7] with the following nested primer sets: No.1 outer 5'-AGCACTCGCAGGGCAGTACG-3' (SEQ ID NO:51); 5'-GACTCTTCGCTGATGAAACTGG-3' (SEQ ID NO:52); No. 1 inner 5'-TCCGTGTTGTCTACGTCCAG-3' (SEQ ID NO:53), 5'-AGCCGAAAGGATTCCACCAT-3' (SEQ ID NO:41); No. 2 outer 5'-AGGCAACGTCAGATGTTGAC-3' (SEQ ID NO:54), 5'-GAAATTACCCACGAGATCGC-3' (SEQ ID NO:42); No. 2 inner 5'-CATGGGAGTACATTGTCAGGACCTC-3' (SEQ ID NO:55), 5'-GGAATTATCTCGCAGGTTGCC-3' (SEQ ID NO:56); No. 3 outer 5'-GGCGACATTCATCAACCTCAGGG-3' (SEQ ID NO:57), 5'-ATATCATCCTGTGCGTTCACGAC-3' (SEQ ID NO:58); No. 3 inner 5'-CATGGGAGTACATTGTCAGGACCTC-3' (SEQ ID NO:55), 5'-GGAATTATCTCGCAGGTTGCC-3 (SEQ ID NO:56). The outer primer set was amplified for 35 cycles at 94^0 C for 30 seconds, 60^0 C for 1 minute and 72^0 C for 1 minute and a 5 minute final extension cycle at 72^0 C. One to three ml of the PCR product was added to the inner PCR reaction mixture and amplified for 25 additional cycles with a 5 minute final extension cycle. Primary determination of sample positivity was made with primer set No. 1 and confirmed with either primer sets 2 or 3 which amplify noneverlapping regions of the KSHV hypothetical major capsid gene. Sampling two portions of the KSHV genome decreased the likelihood of intraexperimental PCR contamination. These nested primer sets are 2-3 longs more sensitive for detecting KSHV sequences than the previously published KS3330$_{233}$ PRIMERS [6] and are estimated to be able to detect <10 copies of KSHV genome under optimal conditions. Sample preparations were prealiquoted and amplified with alternating negative control samples without DNA to monitor and control possible contamination. All samples were tested in a blinded fashion and a determination of the positivity/negativity made

Page 6, lines 9-15:

Comparison of amino acid homologies between EBV ORF BDLF1 (SEQ ID NO: 47), HSVA ORF 26 (SEQ ID NO: 46) and a 918 bp reading frame of the Kaposi's sarcoma agent which includes KS330Bam. Amino acid identity is denoted by reverse lettering. In HSVSA, ORF 26 encodes a minor capsid VP23 which is a late gene product.

Page 8, lines 6-9:

Indirect immunofluorescence end point and geometric mean titers (GMT) in AIDS-KS and AIDS control sera against [BHL-6] HBL-6 and P3H3 prior to and after adsorption with P3H3.

Page 11, lines 9-20:

Representative example of IFA staining of [BHL-6] HBL-6 with AIDS-KS patient sera and control sera from HIV-infected patients without KS. Both AIDS-KS (Figure 19A) and control (Figure 19B) sera show homogeneous staining of [BHL-6] HBL-6 at 1:50 dilution. After adsorption with paraformaldehyde-fixed P3H3 to remove cross reacting antibodies directed against lymphocyte and EBV antigens, antibodies from AIDS-KS sera localize to [BHL-6] HBL-6 nuclei (Figure 19C). P3H3 adsorption of control sera eliminates immunofluorescent staining of [BHL-6] HBL-6 (Figure 19D).

Page 24, lines 24 and 25:

1) AGCCGAAAGGATTCCACCAT; (SEQ ID NO: 41)
TCCGTGTTGTCTACGTCCAG (SEQ ID NO:[41]48)

Page 24, lines 27 and 28:

2) GAAATTACCCACGAGATCGC; (SEQ ID NO: 42)
AGGCAACGTCAGATGTGA (SEQ ID NO:[42]49)

Page 24, lines 30 and 31:

3) AACACGTCATGTGCAGGAGTGAC; (SEQ ID NO:43)
CGGGTGACAGTTGTGATCTAAGG (SEQ ID NO:[43]50)

Page 26, lines 14-20:

One skilled in the art may isolate and propagate the DNA herpesvirus associated with Kaposi's sarcoma (KSHV) employing the following protocol. Long-term establishment of a B lymphoid cell line infected with the KSHV from body-cavity based lymphomas (RCC-1 or [BHL-6] HBL-6) is prepared extracting DNA from the Lymphoma tissue using standard techniques [27, 49, 66].

Page 26, lines 22-32:

The KS associated herpesvirus may be isolated from the cell DNA in the following manner. An infected cell line ([BHL-6] HBL-6 RCC-1), which can be lysed using standard methods such as hyposomatic shocking and Dounce homogenization, is first pelleted at 2000xg for 10 minutes, the supernatant is removed and centrifuged again at 10,000xg for 15 minutes to remove nuclei and organelles. The supernatant is filtered through a 0.45μ filter and centrifuged again at 100,000xg for 1 hour to pellet the virus. The virus can then be washed and centrifuged again at 100,000xg for 1 hour.

Page 101, lines 5-32:

The KS330Bam sequence is an internal portion of an 918 bp ORF with 55-56% nucleotide identify to the ORF26 and BDLF1 genes of HSVSA

and EBV respectively. The EBV and HSVSA translated amino acid sequences for these ORFs demonstrate extensive homology with the amino acid sequence encoded by the KS-associated 918 bp ORF (Figure 6). In HSVSA, the VP23 protein is a late structural protein involved in capsid construction. Reverse transcriptase (RT)-PCR of mRNA from a KS lesion is positive for transcribed KS330Bam mRNA and that indicates that this ORF is transcribed in KS lesions. Additional evidence for homology between the KS agent and herpesviruses comes from a comparison fo the genomic organization of other potential ORFs on the 9404 bp sequence (Figure 3A) The 5' terminus of the sequence is composed nucleotides having 66-67% nucleotide identity and 68-71% amino acid identity to corresponding regions of the major capsid protein (MCP) ORFs for both EBV and HSVSA. This putative MCP ORF of the KS agent lies immediately 5' to the BDLF1/ORF26 homolog which is a conserved orientation amount herpesvirus subfamilies for these two genes. At the 3' end of this sequence, the reading frame has strong amino acid and nucleotide homology to HSVSA ORF 27. Thus, KS-associated DNA sequences at four loci in two separate regions with homologies to gamma herpesviral genomes have been identified.

Page 135, lines 1-11:

results were obtained by using BCBL-1 instead of [BHL-6] HBL-6 cells, by pre-adsorbing with EBV-infected nonproducer Raji cells instead of P3H3 and by using sera from a homosexual male KS patient without HIV infection, in complete remission for KS for 9 months ([BHL-6] HBL-6 titer 1:450, P3H3 titer 1:150). Paired sera taken 8-14 months prior to KS onset and after KS onset were available for three KS patients: KS patients 8 and 13 had eight-fold rises and patient 8 had a three-fold fall in P3H3-adsorbed BCBL-1 titers from pre-onset sera to post-KS sera.

Page 138, lines 24-39:

Infections with the human herpesviruses are generally ubiquaitous in that nearly all humans are infected by early adulthood with six of the seven previously identified human herpesviruses [42]. Universal infection with EBV, for example, is the primary reason for the difficulty in clearly establishing a casual role for this virus in EBV-associated human tumors. The serologic studies identified nuclear antigen in BCBL-1 and [BHL-6] HBL-6 which is recognized by sera from AIDS-KS patients but generally not by sera from control AIDS patients without KS after removal fo EBV-reactive antibodies. These data are consistent with PCR studies of KS and control patient lymphocytes suggesting that KSHV is not ubiquitous among adult humans, but is specifically associated with persons who develop Kaposi's sarcoma. In this respect, it appears to be

Page 147, lines 17-33:

Hemophilic and Homosexual/Bisexual Male AIDS Patient Control Enrollment: Two control groups of AIDS patients were examined: 23 homosexual/bisexual men with AIDS followed until death who did not develop KS ("high risk" control group) from the Multicenter AIDS Cohort Study [16]), and 19 hemophilic men ("lowrisk" control group) enrolled from joint projects of the National Hemophilia Foundation and the Centers for Disease Controls with available follow-up information, none are known to have developed KS and <2% of hemophilic AIDS patients historically develop KS [2]. For homosexual/bisexual AIDS control patients who did not develop KS, paired PBMC specimens were available at entry into their cohort sutdy (median -35 months prior to AIDS onset) and at the study visit immendiately prior to nonKS AIDS diagnosis (median [BHL-6] HBL-6 months prior to AIDS onset).

filtered to form a single cell suspension by standard techniques [49, 66]. The cells are separated by standard Ficoll-Plaque centrifugation and lymphocyte layer is removed. The lymphocytes are then placed at >1x10^6 cells/ml into standard lymphocyte tissue culture medium, such as RMP 1640 supplemented with 10% fetal calf serum. Immortalized lymphocytes containing the KSHV virus are indefinitely grown in the culture media while nonimmortilized cells die during course of prolonged cultivation.

Further, the virus may be propagated in a new cell line by removing media supernatant containing the virus from a continuously infected cell line at a concentration of >1x10^6 cells/ml. The media is centrifuged at 2000xg for 10 minutes and filtered through a 0.45μ filter to remove cells. The media is applied in a 1:1 volume with cells growing at >1x10^6 cells/ml for 48 hours. The cells are washed and pelleted and placed in fresh culture medium, and tested after 14 days of growth.

RCC-1 and RCC-1_{2F5} were deposited on October 19, 1994 under ATCC Accession No. CRL 11734 and CRL 11735, respectively, pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A.

BHL-6 was deposited on November 18, 1994 under ATCC Accession No. CRL 11762 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A.

KS390Bam and KS480Bam hybridized nonspecifically to both KS and non-KS tissues and were not further characterized. 20 of 27 (74%) AIDS-KS DNAs hybridized with variable intensity to both KS330Bam and KS627Bam, and one additional KS specimen hybridized only to KS627Bam by Southern blotting (Figure 2 and Table 1). In contrast to AIDS-KS lesions, only 6 of 39 (15%) non-KS tissues from patients with AIDS hybridized to the KS330Bam and KS627Bam inserts (Table 1).

Specific hybridization did not occur with lymphoma or lymph node DNA from 36 persons without AIDS or with control DNA from 49 tissue biopsy specimens obtained from a consecutive series of patients. DNA extracted from several vascular tumors, including a hemangiopericytoma, two angiosarcomas and a lymphangioma were also negative by Southern blot

Table 1. Southern blot hybridization for KS330Bam and KS627Bam and PCR amplification for $KS330_{234}$ in human tissues from individual patients.

Tissue	n	KS330Bam Southern hybridization n(%)	KS627Bam Southern hybridization n(%)	$KS330_{234}$ PCR positive
AIDS-KS	27*	20 (74)	21 (78)	25 (93)
AIDS lymphomas	27†	3 (11)	3 (11)	3 (11)
AIDS lymph nodes	12	3 (25)	3 (25)	3 (25)
Non-AIDS Lymphomas	29	0 (0)	0 (0)	0 (0)
Non-AIDS lymph nodes	7	0 (0)	0 (0)	0 (0)
Vascular tumors	4§	0 (0)	0 (0)	0 (0)
Opportunistic	13¶	0 (0)	0 (0)	0 (0)

Serologic Studies:

Indirect immunofluorescence antibody assays (IFA) were used to assess the presence of specific antibodies against the KSHV- and EBV-infected cell line BHL-6 in the sera from AIDS-KS patients and control patients with HIV infection or AIDS. BHL-6 was substituted for BCBL-1 for reasons of convenience; preliminary studies showed no significant differences in IFA results between BHL-6 and BCBL-1. BHL-6 have diffuse immunofluorescent cell staining with most KS patient and control unabsorbed sera suggesting nonspecific antibody binding (Figures 19A-19D). After adsorption with paraformaldehyde-fixed, TPA-induced P3H3 (an EBV producer subline of P3J-HR1, a gift of Dr. George Miller) to remove cross-reacting antibodies against EBV and lymphocyte antigens, patient sera generally showed specular nuclear staining at high titers while this staining pattern was absent from control patient sera (Figures 19B and 19D). Staining was localized primarily to the nucleus but weak cytoplasmic staining was also present at low sera dilutions.

With unadsorbed sera, the initial endpoint geometric mean titers (GMT) against BHL-6 cell antigens for the sera from AIDS-KS patients (GMT=1:1153, range: 1:150 to 1:12,150) were higher than for sera from control, non-KS patients (GMT=1:342; range 1:50 to 1:12,150; p=0.04) (Figure 13). While AIDS-KS patients and HIV-infected gay/bisexual and intravenous drug user control patients had similar endpoint titers to BHL-6 antigens (GMT=1:1265 and GMT=1:1578, respectively), hemophilic AIDS patient titers were lower (GMT=1:104). Both case and control patient groups had elevated IFA titers against the EBV infected cell line P3H3.

The difference in endpoint GMT between case and control titers against BHL-6 antigens increased after adsorption with P3H3. After adsorption, case GMT declined to 1:780 and control GMT declined to 1:81 (p=0.00009). Similar

before code breaking. Significance testing was performed with Mantel-Haenszel chi-squared estimates and exact confidence intervals using Epi-Info ver. 6 (USD Inc., Stone Mt.GA).